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Lab Resource: Stem Cell Line

Generation of the human induced pluripotent stem cell (hiPSC) line PSMi002-A from a patient affected by the Jervell and Lange-Nielsen syndrome and carrier of two compound heterozygous mutations on the KCNQ1 gene

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ABSTRACT

We report the generation of human induced pluripotent stem cells (hiPSCs) from dermal fibroblasts of a female patient carrier of the two compound heterozygous mutations c.568 C>T p.R190W (maternal allele), and c.1781 G>A p.R594Q (paternal allele) on the KCNQ1 gene, causing Jervell and Lange-Nielsen Syndrome (JLNS). To obtain hiPSCs, we used the classical approach of the four retroviruses each encoding for a reprogramming factor OCT4, SOX2, KLF4, cMYC. The obtained hiPSC clones display pluripotent stem cell characteristics, and differentiate into spontaneously beating cardiomyocytes (hiPSC-CMs).

Resource table.

Unique stem cell line identifier	PSMi002-A
Alternative name of stem cell line	HDF30-JLNS-iPS
Institution	Fondazione IRCCS Policlinico San Matteo
Contact information of distributor	Massimiliano Gnecchi, m.gnecchi@unipv.it
Type of cell line	hiPSC
Origin	Human
Additional origin info	Age: 10 Gender: female Ethnicity: Caucasian
Cell source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviruses encoding for the human cDNA of OCT4, SOX2, cMYC, KLF4
Genetic modification	No
Type of modification	N/A
Associated disease	Jervell and Lange-Nielsen Syndrome (OMIM #220400)
Gene/locus	568 C>T and 1781 A>G mutations on KCNQ1 (NM_000218.2)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	Jan 7, 2013

(continued on next page)

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Cell line repository/bank	No
Ethical approval	The study has been approved by the Ethics Committee of our Institution, Fondazione IRCCS Policlinico San Matteo, on the 29th of October 2010, protocol number 20100004354, proceeding P-20100003369. We obtained patient written informed consent for both skin biopsy procedure and conservation of biological samples.

Resource utility

It has been proven that iPSCs and iPSC-CMs can be efficiently used to model LQTS, and test targeted therapies (Gnecchi et al., 2017; Mehta et al., 2017; Mura et al., 2017; Rocchetti et al., 2017). The PSMi002-A cell line can be used for: 1) modelling of JLNS; 2) targeted drug testing.

Resource details

The PSMi002-A line was generated by reprogramming of dermal fibroblasts isolated from skin biopsy of a 10 years old girl affected by Jervell and Lange-Nielsen Syndrome (JLNS). JLNS is a recessive form of Long QT Syndrome associated with bilateral hearing loss, marked prolongation of the QT interval at the surface ECG, and a propensity for life-threatening cardiac arrhythmias and sudden cardiac death (Jervell and Lange-Nielsen, 1957). The enrolled patient has a severe, bilateral sensorineural hearing loss, a significantly prolonged QTc (QT corrected for heart rate of 578 ms), and experienced several syncopal episodes since age 2. At 7 years the diagnosis of JLNS was performed, beta-blocker therapy was started and no other cardiac events occurred. The patient carries two compound heterozygous mutations on the KCNQ1 gene: the c.568 C/T on the maternal allele leads to the substitution of the arginine in position 190 with tryptophan; the c.1781 G/A on the paternal allele leads to the substitution of the arginine in position 594 with a glutamine.

Fibroblasts were reprogrammed by retroviral infection of OCT4, SOX2, KLF4 and c-MYC. The obtained hiPSCs were maintained on feeders, retaining ES-like morphology and pluripotent features up to passage 50. Both fibroblasts and hiPSCs present the disease causing mutations on the KCNQ1 gene, as proved by DNA sequencing (Fig. 1A. The KCNQ1 coding sequence -CDS- used as a reference is the NCBI sequence NM_000218.2) and an identical DNA profile at seven polymorphic loci, as shown by Short tandem Repeat (STR) analysis (submitted in archive with journal). Moreover, the DNA karyotyping revealed normal female karyotype (46, XX) (Fig. 1B). The PSMi002-A uniformly expresses the human ES surface antigens Tumor Related Antigen-1-60 and -1-81 (TRA-1-60 and TRA-1-81), Stage Specific Embryonic Antigen-3 and -4 (SSEA-3, SSEA-4), and shows alkaline phosphatase (AP) activity (Fig. 1C). Likewise, it expresses the pluripotent markers NANOG, OCT4, SOX2 (Fig. 1C and D), REX1, GDF3, ESG1, DPPA2, DPPA4 and NODAL (Fig. 1D), and shows OCT4 promoter demethylation (Fig. 1E, open circles indicate unmethylated CpG dinucleotides, while closed circles indicate methylated CpGs). RT-PCR analysis in Fig. 1F shows no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), clear expression of Tg OCT4, SOX2, KLF4 and cMYC in fibroblasts five days after transduction (OSKM) and silencing of the four Tg in the PSMi002-A.

As expected, PSMi002-A spontaneously forms embryoid bodies (EBs) able to differentiate into cells belonging to the three germ layers: endoderm, mesoderm and ectoderm (Fig. 1G). Most importantly, we have successfully differentiated this JLNS cell line into cardiomyocytes displaying spontaneous beating activity, and expressing the sarcomeric proteins alpha-actinin (α-SA) and troponin T (TnT) (Fig. 1H, the insets show areas of cross-striation). We also verified the absence fo mycoplasma contamination in our PSMi002-A line (Fig. 1I).

Materials and methods

Generation and clonal expansion of hiPSCs

The detailed protocol is provided as Supplemental methods.

Briefly, skin fibroblasts were reprogrammed using four retroviral vectors expressing OCT4, SOX2, KLF4 and cMYC. Clonal selection of fully reprogrammed cells was performed manually under sterile conditions and using an EVOS XL Core Imaging System (ThermoFisher), by picking individual clones morphologically similar to embryonic stem cells. Colonies were cut, harvested with a pipette, individually placed into a separate cell culture well and expanded (Table 1).

Mutation analysis

Genomic DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen). KCNQ1 gene sequence was amplified with the GoTaq G2 DNA polymerase (Promega) (see Table 2 for primer sequences). The resulting amplicons were purified and sequenced (Lightrun service - GATC Biotech AG – Germany).

STR analysis

STR analysis was carried out using PowerPlex® CS7 System (Promega) kit, following the manufacturer's protocol. Fragments were run on a 3130xl capillary sequencer (Applied Biosystems). Genotypes were assigned using GeneMarker software (SoftGenetics).

Karyotyping

hiPSCs were blocked at metaphase by exposition to 10 μg/ml demecolcine solution (Sigma Aldrich) for 3 h. Karyotyping was performed using 300 G-banding chromosome analysis.

Fig. 1. Characterization of the PSMi002-A cell line. A. On top schematic representation of the KCNQ1 gene with introns (horizontal lines) and exons (vertical lines/boxes). In the lower panel, DNA sequencing results showing the presence of the mutations 568 C>T and 1781 G>A in the KCNQ1 gene of patient-derived dermal fibroblasts (HDF) and PSMi002-A cell line (hiPSC) derived from the same HDF. The KCNQ1 coding sequence (CDS) used as a reference is the NCBI sequence NM_000218.2. B. Karyotype analysis of PSMi002-A (300 G-bandings) showing normal female karyotype (46, XX). C. Immunofluorescence staining showing uniform expression of the indicated markers of pluripotency in the PSMi002-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). The AP panel reports an alkaline phosphatase colorimetric staining. D. RT-PCR analysis showing expression of the indicated markers of pluripotency in PSMi002-A (hiPSC), compared with their parental fibroblasts (HDF). E. OCT-4 promoter methylation analysis with bisulfite sequencing in patient's dermal fibroblasts (HDF) and in the derived hiPSCs. Open circles indicate unmethylated CpG dinucleotides, while closed circles indicate methylated CpGs. F. RT-PCR analysis showing no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), expression of Tg OCT4, SOX2, KLF4 and cMYC five days after transduction (OSKM) and silencing of the four Tg in PSMi002-A. G. Far left panel: floating embryoid bodies (EBs) formed after 7 days of PSMi002-A culture in suspension. Panels on the right: Immunofluorescence staining for markers of the 3 germ layers in iPSC-derived EBs: neuronal class tubulin beta III (Tuj) and microtubule-associated protein 2 (MAP2) for ectoderm, smooth muscle actin (SMA)) and cardiac troponin I (TnI) for mesoderm, and alpha Fetoprotein (AFP) for endoderm. H. Co-immunofluorescence staining for the cardiac sarcomeric proteins alpha-sarcomeric actinin (α-SA, red) and troponin T (TnT, green) in cardiomyocytes differentiated from the PSMi002-A. Nuclei were counterstained with Hoechst. The insets show areas of cross-striations. I. EZ-PCR test showing the absence of mycoplasma contamination in PSMi002-A. Ctrl+ is the positive PCR control provided by the kit.

Immunocytochemistry

hiPSCs and their derivatives were grown on glass coverslips, and then fixed for 15 min in 4% paraformaldehyde (Affimetrix USB), permeabilized with 0.1% Triton X-100 (Sigma Aldrich) for 5 min, and blocked in 1% bovine serum albumin (BSA, Sigma Aldrich) for 1 h at room temperature (RT). Then they were incubated for 1 h at RT with the primary antibody (Table 2) diluted in blocking solution,

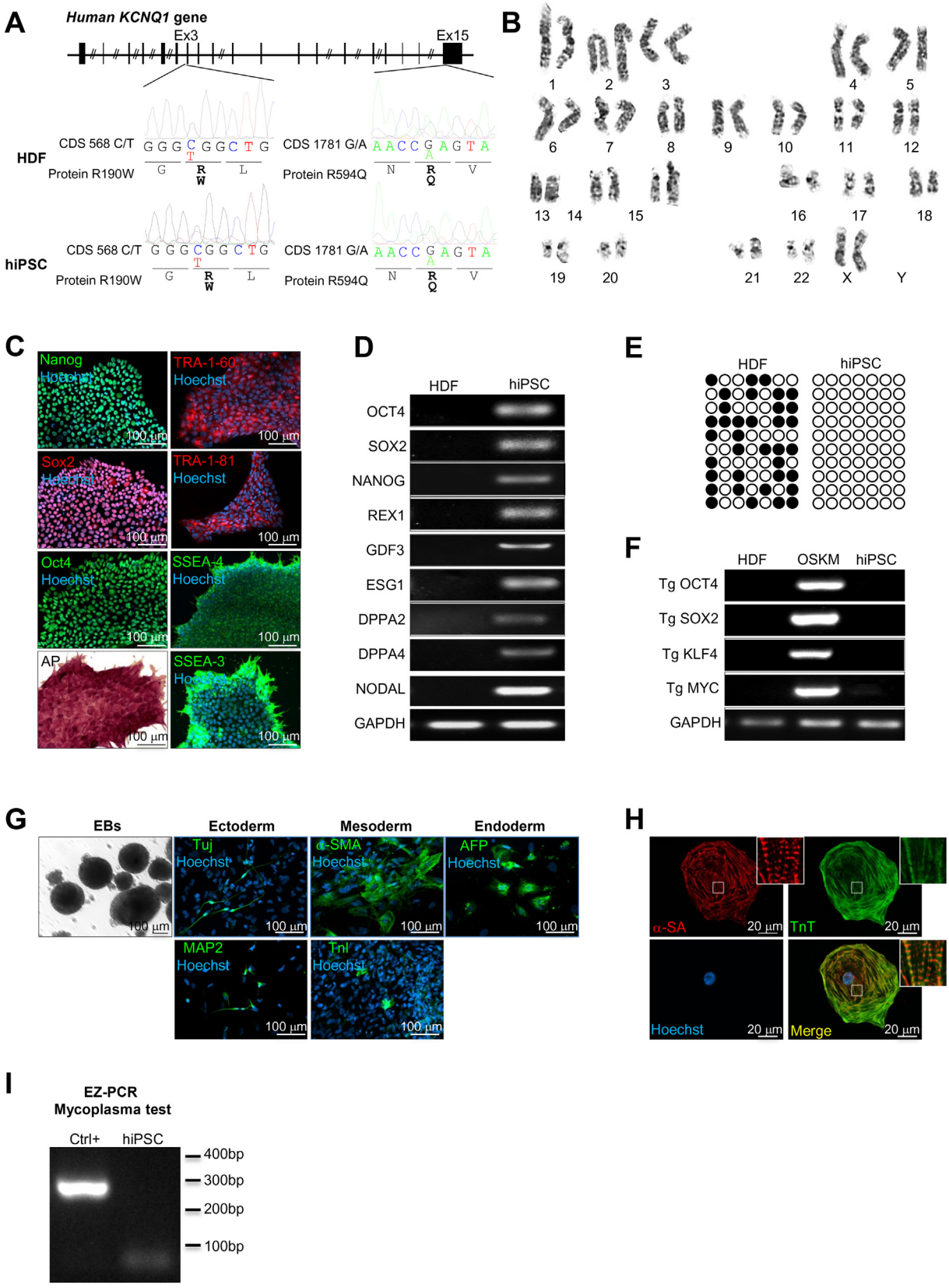

Table 1
Characterization and validation of PSMi002-A cell line.

Classification	Test	Result	Data
Morphology	Photography	Normal	Not shown but available upon request
Phenotype	Immunocytochemistry	Positive staining for the pluripotency markers Oct4, Nanog, Sox2, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4	Fig. 1 panel C
	Alkaline phosphatase assay	Positive staining for the alkaline phosphatase	Fig. 1 panel C
	RT-PCR	Expression of the pluripotency markers OCT3/4, SOX2, NANOG, REX1, GDF3, ESG1, DPPA2, DPPA4, NODAL	Fig. 1 panel D
Genotype	Karyotype (300 G-banding) and resolution	46XX, Resolution 450–500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	7 sites tested for iPSC, all sites matched with donor HDF STR profile	Online archive
Mutation analysis	Sequencing	Compound heterozygous for the mutations c. 568 C>T p.R190W and 1781 G>A p.R594Q on the KCNQ1 gene	Fig. 1 panel A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative	Fig. 1 panel I
Differentiation potential	Embryoid body formation	The EBs expressed neuronal class tubulin beta III (Tuj) and microtubule-associated protein (MAP) (ectoderm); smooth muscle actin (SMA) and troponin I (mesoderm); alpha fetoprotein (AFP) (endoderm).	Fig. 1 panel G
	Differentiation into cardiomyocytes	The iPSC-derived cardiomyocytes expressed the cardiac sarcomeric proteins alpha-sarcomeric actinin (α-SA) and troponin T (TnT)	Fig. 1 panel H
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	
Genotype additional info	Blood group genotyping	Not performed	
	HLA tissue typing	Not performed	

washed three times, and incubated for 1 h at RT with an appropriate secondary antibody (Table 2). Finally, the cells were stained with 1 μg/ml of Hoechst 33258 (Sigma Aldrich). Images were acquired using the Carl Zeiss fluorescence microscope Observer.Z1 equipped with the Apotome system and AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany).

AP colorimetric assay

AP was detected by using the Alkaline Phosphatase Staining kit (00-0009 Stemgent).

RT-PCR

Total RNA was purified using TRIzol (ThermoFisher Scientific). cDNA was synthesized using the Superscript II Reverse Transcriptase (ThermoFisher). RT-PCR was performed with the GoTaq G2 DNA polymerase (Promega) and primers in Table 2.

OCT4 promoter demethylation analysis

Genomic DNA was treated with the EZ DNA methylation kit (Zymo Research, Orange, CA, USA). The promoter region of the human OCT4 gene was amplified using Amplitaq gold 360 (Applied Biosystems). The PCR products were sequenced using Pyrosequencing PSQ96 HS System (Biotage, Uppsala, Sweden). The methylation status of each locus was analyzed using PyroQ-CpG software (Qiagen).

EB formation

hiPSCs were grown for 7 days in non-adherent conditions in a modified iPS medium deprived of bFGF and containing 20% FBS instead of KO-SR. Forming EBs were then transferred to gelatin-coated dishes to allow differentiation in adhesion for additional 7 days. Finally, the cells were processed for immunostaining of the three germ layers as described above.

Cardiac differentiation

Cardiac differentiation was induced using the PSC Cardiomyocyte Differentiation Kit (ThermoFisher).

Mycoplasma test

For the detection of mycoplasma in cell culture we used the EZ-PCR Mycoplasma Test Kit (Biological Industries).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.04.002.

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Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti Nanog	1:200	Stemgent Cat# 09-0020, RRID: AB_2298294
	Mouse anti Oct3/4 (C-10)	1:500	SCBT Cat# sc-5279, RRID: AB_628051
	Mouse anti Sox2	1:500	R&D Systems Cat# MAB2018, RRID: AB_358009
	Mouse anti TRA-1-60	1:100	Stemgent Cat# 09-0010, RRID: AB_1512170
	Mouse anti TRA-1-81	1:100	Stemgent Cat# 09-0011, RRID: AB_1512171
	Rat anti SSEA-3	1:100	Millipore Cat# MAB4303, RRID: AB_177628
	Mouse anti SSEA-4	1:100	Stemgent Cat# 09-0006, RRID: AB_1512169
Differentiation markers (EBs)	Mouse anti neuronal class tubulin beta III (Tuj)	1:500	Covance Cat# MMS-435P, RRID: AB_2313773
	Anti microtubule-associated protein 2 (MAP2)	1:200	Millipore Cat# MAB3418, RRID: AB_94856
	Mouse anti smooth muscle actin	1:1000	Millipore Cat# CBL171, RRID: AB_2223166
	Mouse anti Troponin I (TnI)	1:200	Millipore Cat# MAB1691, RRID: AB_2256304
	Mouse anti alpha-fetoprotein	1:500	Millipore Cat# SCR030, RRID: AB_597591
Cardiac markers	Mouse anti Troponin T[a]	1:250	ThermoFisher Cat# MA5-12960, RRID: AB_11000742
	Mouse anti alpha actinin[a]	1:800	Sigma Aldrich Cat# A7811, RRID: AB_476766
Secondary antibodies	Alexa-Fluor® 488 Goat anti-rabbit IgG	1:500	ThermoFisher Cat# A11008, RRID:AB_143165
	Alexa-Fluor® 488 Goat anti-rat IgM	1:500	ThermoFisher Cat# A21212, RRID:AB_11180047
	Alexa-Fluor® 488 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11001, RRID: AB_2534069
	Alexa-Fluor® 546 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11003, RRID: AB_141370

Primers		
	Target	Forward/reverse primer (5′–3′)
Targeted mutation analysis/sequencing	KCNQ1 exon 3	Fw: 5′-gttcaaacaggttgcagggtctga-3′ Rev: 5′- ccaggtttccagaccaggaag-3′
	KCNQ1 exon 15	Fw: 5′-ctacctcccccagccctac-3′ Rev: 5′-caactcccaagaggggcc-3′
Pluripotency markers (RT-PCR)	OCT4	Fw: 5′-gtactcctcggtccctttcc-3′ Rev: 5′-caaaaaccctggcacaaact-3′
	SOX2	Fw: 5′-acaccaatcccatccacact-3′ Rev: 5′-tttttcgtcgcttggagact-3′
	NANOG	Fw: 5′-ttccttcctccatggatctg-3′ Rev: 5′-tctgctggaggctgaggtat-3′
	REX1	Fw: 5′-cagatcctaaacagctcgcagaat-3′ Rev: 5′-gcgtacgcaaattaaagtccaga-3′
	GDF3	Fw: 5′-cttatgctacgtaaaggagctggg-3′ Rev: 5′-gtgccaacccaggtcccggaagtt-3′
	ESG1	Fw: 5′-atatcccgccgtgggtgaaagttc-3′ Rev: 5′-actcagccatggactggagcatcc-3′
	DPPA4	Fw: 5′-ggagccgcctgccctggaaaattc-3′ Rev: 5′-tttttcctgatattctattcccat-3′
	DPPA2	Fw: 5′-ccgtccccgcaatctccttccatc-3′ Rev: 5′-atgatgccaacatggctcccggtg-3′
	NODAL	Fw: 5′-gggcaagaggcaccgtcgacatca-3′ Rev:5′-gggactcggtggggctggtaacgtttc-3′
House-keeping genes (RT-PCR)	GAPDH	Fw 5′-catgttccaatatgattccaccc-3′ Rev. 5′-gggatctcgctcctggaagat-3′
OCT4 promoter demethylation analysis/bisulfite sequencing	OCT4 promoter	Fw: 5′-gaggttggagtagaaggattgttttggttt-3′ Rev: 5′-ccccctaacccatcacctccaccacctaa-3′
Retroviral transgenes	OCT4 cDNA on pMXs-hOCT3/4	Fw: 5′-ccccagggccccattttggtacc-3′
	SOX2 cDNA on pMXs-hSOX-2	Fw: 5′-ggcacccctggcatggctcttggctc-3′
	cMYC cDNA on pMXs-hcMYC	Fw: 5′-caacaaccgaaaatgcaccagccccag-3′
	KLF4 cDNA on pMXs-hKLF4	Fw: 5′-acgatcgtggccccggaaaaggacc-3′
	pMX viral vector	Rev: 5′-ccctttttctggagactaaataaa-3′

[a] To perform the co-staining with these two antibodies, we used the Zenon Tricolor Mouse IgG labeling Kit (Molecular Probes).